

ments, groups of 10 C57BL/6 mice were injected (i.p.) with 10 mg/kg of a given compound 1 hour before and on days 2 and 5 post challenge with 1000 pfu of mouse adapted EBOV (m-EBOV). See Bray et al. (1999) *J. Infect. Dis.* 179 (Suppl 1):S248-58, which is herein incorporated by reference. Control mice were injected with saline. All of the mice were injected (i.p.) with 1000 pfu virus each. Food and water were provided to the mice and the mice were monitored for at least 14 days post challenge. FIG. 6 shows the percent survival. As shown in FIG. 6, NSC 369723 and NSC 294202 provided 100% protection and 90% of the control mice died from infection.

[0173] The antiviral activity of various doses of NSC 306365 post exposure was examined. In these experiments, groups of 10 C57BL/6 mice were challenged with 1000 pfu of m-EBOV each. After 24 hours post challenge, the mice of each group were injected (i.p.) with a given dose of NSC 306365. Food and water were provided to the mice and the mice were monitored for at least 14 days post challenge. As shown in FIG. 7, a 5 mg/kg dose of NSC 306365 conferred 100% protection against infection and doses as low as 0.5 mg/kg provided 60% protection.

4. In Vivo Assays—Marburg Virus

[0174] The antiviral activities of NSC 369723, NSC 294202, NSC 306365, and NSC 300510 were tested against Marburg virus infection in BALB/C mice. In these experiments, groups of 10 BALB/C mice were injected (i.p.) with 5 mg/kg of NSC 306365 or 10 mg/kg of NSC 369723, NSC 294202, or NSC 300510 1 hour before and on days 2 and 5 post challenge with 1000 pfu of mouse-adapted Marburg Ravn virus (R-MARV). See Warfield et al. (2007) *Virol. J.* 4:108, which is herein incorporated by reference and below. Control mice were injected with saline. All of the mice were injected (i.p.) with 1000 pfu virus each. Food and water were provided to the mice and the mice were monitored for at least 14 days post challenge. FIG. 8 shows the percent survival. As shown in FIG. 8, except for NSC 306365, NSC 369723, NSC 294202, and NSC 300510 provided 100% protection against m-MARV.

[0175] The mouse-adapted Marburg Ravn virus (R-MARV) was generated by serial passage of the virus in liver homogenates 25× through severe combined immunodeficient (scid) and then BALB/c mice. Serially passaging the livers from MARV-infected scid mice has been highly successful in reducing the time to death in scid mice from 50-70 days to 7-10 days following MARV-Ci67, -Musoke, or -Ravn challenge. See Warfield et al. (2007) *Virol. J.* 4:108, which is herein incorporated by reference. Further, sequential passages in BALB/c mice allowed the MARV to cause lethality in both BALB/c and C57BL/6 mice. Serial sampling studies to characterize the pathology of the mouse-adapted MARV-Ravn revealed that the mouse-adapted MARV model has many similar properties as the guinea pigs and primate MARV models. Infection of BALB/c mice with mouse-adapted MARV-Ravn caused uncontrolled viremia ($>10^6$ pfu/ml), extremely high viral titers in the liver, spleen, lymph node and other organs, profound lymphocytopenia and destruction of lymphocytes within the spleen and lymph nodes, and marked liver damage.

5. Pre- and Post-Exposure Efficacy

[0176] The pre- and post-exposure efficacy of NSC 300510 and post exposure efficacy of NSC 369723 and NSC 294199

were examined against EBOV infection in mice. In these experiments, Group 1 of 10 BALB/C mice were injected (i.p.) with 10 mg/kg of a NSC 300510 1 hour before challenge with 1000 pfu of mouse adapted EBOV (m-EBOV). The remaining groups, Groups 2-5, of mice were injected with saline. On day 1 after challenge, Group 1 was injected with saline, group 2 with NSC 300510, group 3 with NSC 294199, group 4 with NSC 369723, and group 5 with saline. On day 2 after injection, group 1 was injected with NSC 300510. On day 5 after injection all the groups were injected again with the same compounds as administered before. Thus the first group received NSC 300510 on days 0, 2, 5 (pre and post exposure), and the groups 2-4 received the respective compounds only post exposure (days 1 and 5 after challenge).

[0177] Food and water were provided to the mice and the mice were monitored for at least 14 days post challenge. Percent survival on each day was calculated and plotted. As shown in FIG. 9, when administered on days 0, 2, and 5, NSC 300510 protected 100% of mice. When the treatment was delayed to 24 hours after infection, 90% of treated mice survived. In the control group, all became ill and only 30% survival was observed.

6. Cellular Action

[0178] A. To determine if pre-treatment of cells with NSC 369723 increases their antiviral activity, i.e. induces an antiviral state in the cells, the following experiment was conducted. Vero E6 cells were plated on 96 well assay plates (5×10^4 cells/well) in 100 μ l cEMEM medium. The cells were cultured for 3 days at 37° C. in a humidified incubator (5% CO₂). Media were removed from the wells of the 96 well plates and replaced by 100 μ l of media containing 10 μ M NSC 369723 including a control containing no compound. Each compound concentration was repeated in three wells (triplicate). The cells were then incubated again in the incubator at 37° C. for an additional 18 to 24 hours. Three wells received medium containing no drug as control. The experimental wells were as follows:

[0179] Wells 1-3: medium only

[0180] Wells 4-6: 10 μ M 369723 in cEMEM medium

[0181] Wells 7-9: medium only

[0182] Then the media in wells 1-9 was removed and cells were washed with 200 μ l PBS three times and 100 μ l fresh medium without compound was added to wells 1-6. 100 μ l of 10 μ M NSC 369723 was added to the wells 7-9. After adding 50 μ l of GFP-EBOV (10^6 infectious virus particles per ml) to each well, the cells were incubated at 37° C. for 1 hour. Then the media in wells 1-9 was removed and the cells were washed with 200 μ l PBS three times. 100 μ l fresh medium without compound was added to wells 1-6. 100 μ l of 10 μ M NSC 369723 was added to the wells 7-9. The cells were incubated at 37° C. for an additional 40 to 48 hours. Thus, the cells in wells 1-3 did not receive compound at any time, cells in wells 4-6 were treated with compound only before infection, and the cells in wells 7-9 were not pretreated with the compound, but treated during and after infection.

[0183] Then the media was aspirated and the assay plates with the cells were submerged in 4% formaldehyde in PBS buffer in plastic bags (one assay plate per bag) to inactivate the virus. The bags were sealed and incubated at room temperature for three days. Then, the formaldehyde solution was removed from the assay plates and replaced with 100 μ l of PBS/well. The assay plates were then subjected to high throughput screening as described above to measure the per-